GENETIC VARIABILITY AND FUMONISIN B1 PRODUCTION OF FUSARIUM PROLIFERATUM AND FUSARIUM VERTICILLIOIDES ASSOCIATED WITH DISEASED PLANTS

NAJIHAH BINTI AZMAN

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By

NAJIHAH BINTI AZMAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

May 2018

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

GENETIC VARIABILITY AND FUMONISIN B_1 PRODUCTION OF *FUSARIUM PROLIFERATUM* AND *FUSARIUM VERTICILLIOIDES* ASSOCIATED WITH DISEASED PLANTS

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MAY 2018

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Several *Fusarium* species are plant pathogen that can cause various diseases on fruits and vegetables. The common *Fusarium* species that can infect plants are *Fusarium proliferatum* and *Fusarium verticillioides*. Ear rot on maize, Fusarium wilt on cucurbits, fruit rot on tomato and banana are diseases caused by these two species. Both species are able to produce fumonisin B_1 (FB_1) that are harmful towards human and animal and currently there is a lack of information regarding the effect from exposure of FB_1. Thus, the objectives of this study were to identify *F. proliferatum* and *F. verticillioides* based on species-specific primers using polymerase chain reaction (PCR), to evaluate the genetic diversity between both species based on microsatellite marker, to detect the production of FB_1 by both species and to examine embryotoxicity effect of FB_1 on the larvae of zebrafish (*Danio rerio)*. Fifty isolates of *Fusarium* species were obtained from different hosts throughout Malaysia. All isolates were identified using species-specific primers of which 29 isolates were identified as *F. proliferatum* and 21 isolates as *F. verticillioides*. The genetic diversity of the isolates was evaluated using six established microsatellite primers. Five out of six primers amplified polymorphic bands with primers showed high number of alleles were (AG)7C and (TCC)5. Meanwhile one primer (TTTC)4 gave negative result with no band amplified. The constructed phylogenetic tree showed two different clades distinguished between *F. proliferatum* and *F. verticillioides* and high diversity among the clades according to different hosts and locality. Successful amplification of the *FUM1* gene showed the presence of this gene in the genome of 48 out of 50 isolates. *Fusarium proliferatum* produced FB_1 ranged from 16.48-6677.32 ppm. Meanwhile *F. verticillioides* produced FB_1 ranged from 12.26-954.01 ppm. From the assessment of embryotoxicity test of FB_1 on larvae

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of zebrafish, five concentrations of FB_1 (0.43, 0.58, 0.72, 0.87 and 1.00 ppm) were tested. Morphological changes of heart, yolk sac and spine of the FB_1 exposed-larvae were observed at 24 to 168 hours post-fertilization (hpf). The mortality rate and abnormality of zebrafish larvae's were significantly increased at 144 hpf exposure. Pericardial edema and yolk sac edema was severely observed at concentration 0.72–1.00 ppm at 120 hpf exposure meanwhile spinal curvature can be observed at all concentrations at 168 hpf. Spontaneous tail coiling showed significant difference to the normal whereas no significant differences on the heart rate. As a conclusion, *F. proliferatum* and *F. verticillioides* can be identified by using species-specific primers. These two species have showed high diversity among the isolates based on microsatellite and phylogenetic analyses.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Science

KEPELBAGAIAN GENETIK DAN PENGHASILAN FUMONISIN B_1 OLEH *FUSARIUM PROLIFERATUM* DAN *FUSARIUM VERTICILLIOIDES* BERKAITAN DENGAN POKOK BERPENYAKIT

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Beberapa spesis *Fusarium* adalah patogen tumbuhan yang menyebabkan pelbagai jenis penyakit pada buah-buahan dan sayur-sayuran. Antara spesis *Fusarium* yang lazim menyebabkan penyakit pokok adalah *Fusarium proliferatum* dan *Fusarium verticillioides*. Penyakit reput tongkol jagung, penyakit layu mentimun dan penyakit reput tomato dan pisang adalah contoh penyakit disebabkan oleh dua spesis ini. Spesis ini mampu menghasilkan Fumonisin B_1 (FB_1) yang merbahaya terhadap manusia dan haiwan dan pada masa kini maklumat mengenai kesan daripada pendedahan FB_1 adalah tidak mencukupi. Objektif kajian ini adalah untuk mengenal pasti *F. proliferatum* dan *F. verticillioides* berdasarkan analisis pencetus sepsis-spesifik rantai polimerase, untuk menilai kepelbagaian genetik kedua-dua spesis berdasarkan analisis mikrosatelit, untuk mengesan penghasilan FB_1 oleh kedua-dua spesis tersebut dan untuk menilai kesan toksin FB_1 terhadap larva ikan zebra (*Danio rerio*). Lima puluh isolat spesis *Fusarium* terlebih dahulu dipencilkan dari pelbagai perumah yang diperoleh dari serata Malaysia. Kesemua pencilan telah dikenalpasti menggunakan pencetus spesis-spesifik, dimana 29 pencilan telah dikenal pasti sebagai *F. proliferatum* dan 21 pencilan telah dikenal pasti sebagai *F. verticillioides.* Kepelbagaian genetik telah dinilai melalui analisis mikrosatelit menggunakan enam pencetus mikrosatelit. Lima daripada enam pencetus tersebut menghasilkan jalur polimorfik di mana pencetus yang paling tinggi penghasilan alel adalah (AG)7C dan (TCC)5 manakala satu pencetus (TTTC)4 memberikan hasil negatif dan tiada amplifikasi. Pohon filogenetik yang dijana menunjukkan dua klad yang membezakan di antara *F. proliferatum* dan *F. verticillioides* berdasarkan kepada perbezaan perumah dan lokasi. Keupayaan mengamplifikasi *FUM1* menunjukkan kewujudan gen ini di dalam 48 daripada 50 isolat. *Fusarium proliferatum* menghasilkn FB_1 pada kadar 16.48–6677.32 ppm manakala *F. verticillioides* menghasilkan FB_1 pada kadar 12.26–954.01 ppm. Berdasarkan penilaian kesan toksin FB_1 terhadap *Danio rerio*, lima kepekatan FB_1 (0.43, 0.58, 0.72, 0.87 and 1.00 ppm) telah

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digunakan. Perubahan morfologi jantung, kantung yolk dan tulang belakang larva yang terdedah kepada FB_1 diperhatikan pada 24 hpf hingga 168 hpf. Kadar kematian dan morfologi tidak normal dilihat mempunyai peningkatan perbezaan yang bererti pada pendedahan 144 hpf. Perkardial edema dan kantung yolk edema dilihat mempunyai kesan teruk pada kepekatan 0.72-1.00 ppm pada 120 hpf manakala lengkungan tulang belakang diperhatikan pada kesemua kepekatan FB_1 pada 168 hpf. Pusingan ekor rawak menunjukkan perbezaan bererti manakala tiada perbezaan bererti pada kadar degupan jantung. Kesimpulannya, *F. proliferatum* dan *F. verticillioides* boleh dikenalpasti dengan menggunakan analisis amplifikasi spesis-spesifik. Kedua-dua spesis menunjukkan kepelbagaian yang tinggi di antara isolat berdasarkan analisis mikrosatelit dan pohon filogenetik.

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
bpm	beats per minute
DSI	Disease Severity Index
EtBr	Ethidium Bromide
FAO	Food and Agricultural Organization
FB_1	Fumonisin B_1
FB_2	Fumonisin B_2
FB_3	Fumonisin B_3
hpf	hours-post fertilization
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ISSRs	Inter-Simple Sequence Repeats
ITS	Internal Transcribed Spacer
LC-MS	Liquid Chromatography Tandem Mass Spectrometry
LEM	Leukoencephalomalacia
OPA	Ophthaldialdehyde
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction

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PCR-RFLP	PCR-Restriction Fragment Length Polymorphism
PDA	Potato Dextrose Agar
PES	Pulmonary Edema Syndrome
PKS	Polyketide Synthase
ppm	part per million
RAPD	Random Amplified Polymorphic DNA
rpm	revolution per minute
SNA	Spezieller Nahrstroffarmer Agar
SSM	Slipped Strand Mispairing
SSRs	Simple Sequence Repeats
STMS	Sequence-Tagged Microsatellite Sites
STS	Sequence-Tagged Sites
TBE	Tris-Borate EDTA
TEF-1α	Translation Elongation Factor-1α
UFLC	Ultra-Fast Liquid Chromatography
UPGMA	Unweighted Pair Group with Arithmetic Mean
UPLC	Ultra-Performance Liquid Chromatography
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

Mycotoxins contamination occurs frequently in the area with hot and humid climate where it is the most suitable condition for growth of fungi. Factors that influence the contamination of mycotoxins in food and feedstuff are related to favorable environmental condition such as unsterile storage area. Proper storage management can control this condition, however, other factors such as climate, fungal strain specificity, strain variation and instability of toxigenic properties are more challenging to handle and control (Zain, 2011).

One of the most common mycotoxins are fumonisins produced by *Fusarium* species. At least 10 different species of *Fusarium* can produce these toxins, however, *Fusarium proliferatum* and *Fusarium verticillioides* are the most common fungi associated with crops, including maize and the highest reported as fumonisins producer (WHO/FAO, 2001). Maize is one of the crops that are subjected to the most critical mycotoxin problems throughout the world. The contamination may occur in the field during storage, processing or feeding under appropriate environmental conditions (Reddy & Salleh, 2011). Apart from maize, there are also various food products that are known to contain a significant amount of fumonisins. The fumonisins-contaminated products include rice, wheat, cowpeas, sorghum, millet, and asparagus (Kushiro, Nagata, Nakagawa & Nagashima, 2008). Other crops that are fumonisins-contaminated including garlic, soybean and pineapple (Palmero et al., 2012; Garcia et al., 2012).

Fusarium species can cause various types of diseases such as root, stem and ear rots, wilt and abnormal growth on many crops and plants. The species in the section Liseola have been reported as potential pathogens of Fusarium ear rot on maize (*Zea mays*), fruit rot on tomato (*Solanum lycopersicum*), wilt on luffa (*Luffa acutangula*) and pumpkin (*Cucurbita pepo)*. These diseases were caused by infection of *F. verticillioides, F. proliferatum* and *F. subglutinans* (Nur Ain Izzati, Siti Nordahliawate, Nor Azlina, Azmi & Baharuddin, 2011a). *Fusarium verticillioides* and *F. proliferatum* can produce significant amount of fumonisins in the infected plants. Fumonisin is a type of mycotoxins and Fumonisin B_1 (FB_1) can interfere with sphingolipid metabolism (Richard et al., 2007). Besides that, both species can also yield other secondary metabolites such as moniliformin, beauvericin and fusaproliferin (Summerell, Baharuddin & Leslie, 2003).

Production of FB_1 by both species depend on a biosynthetic gene cluster *FUM,* which consists of *FUM1* to *FUM19* where *FUM1* are responsible for the production of FB_1 (Glenn et al., 2008). FB_1 is classified in Group 2B

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carcinogens by the International Agency for Research on Cancer (IARC). FB_1 is toxic and causing leukoencephalomalacia and has been reported to be associated with human oesophageal cancer and birth defects (Marasas, 2001).

Maheshwar and Janardhana (2010) reported that 65 isolates of *F. proliferatum* and 27 isolates of *F. verticillioides* were isolated from paddy in India and positive with *FUM1* gene analysis. A study by Chehri, Jahromi, Reddy, Abassi and Baharuddin (2010a) on wheat grains that were marketed in Iran showed that 68.2% of samples were contaminated with FB_1, 42.6% of samples were contaminated with Fumonisin B_2 (FB_2) and 31.7% of samples were contaminated with Fumonisin B_3 (FB_3). Another study in Iran, 51 isolates of *F. verticillioides* and *F. proliferatum* were evaluated using High-Performance Liquid Chromatography (HPLC) have confirmed to produce FB_1, FB_2, and FB_3 from maize cultures (Ghiasian et al., 2005).

The occurrence of mycotoxins has become a great concern as presence of mycotoxins in foods is often associated with acute and chronic diseases (Logrieco, Mule, Moretti & Bottalico, 2002). Food contamination that was caused by toxicants such as mycotoxins can compromise the safety of food and feed supplies to humans and animals. High levels of mycotoxins accumulation have been reported in pre-harvest maize in Europe, North and South America and Asia (Logrieco et al., 2002). Ingestion of certain level of mycotoxins, for example, can result in aflatoxicosis, or ruminant liver failure (Mukanga, Derera, Tongoona & Laing, 2010).

According to European Commission Regulation No. 1881/2006 and European Commission Recommendations 2006/576/EC and 2013/165/EU, the maximum level of FB_1 in unprocessed maize for human consumption is 4 ppm meanwhile for feeding stuff for ruminants is 50 ppm. In 2002, the IARC has evaluated FB_1 originated from *F. verticillioides* classified in Group 2B which means a potential human carcinogen (Kushiro et al., 2008). Since *Fusarium* species can produce toxins that have potential toxicity to humans and domesticated animals, control of mycotoxins contamination has become an urgency in food safety. Fumonisins can be detected symptomatically and asymptomatically and there are some guidelines for maximum fumonisins level in food for human consumption (Brown, Cleveland, Woloshuk, Payne & Bhatnagar, 2001).

The most common and conventional method to detect fumonisins level is using chromatographic method such as HPLC, Ultra-Fast Liquid Chromatography (UFLC) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS). HPLC is practical for routine analysis due to its wider prevalence while LC-MS is practical for screening and quantitative analysis of samples with low contamination levels (Kushiro et al., 2008).

As mycotoxins contamination in daily foods and crops production has become serious problems worldwide, it is important to conduct this study in order to

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detect the production of FB_1 produced by *F. proliferatum* and *F. verticillioides*. Currently Asia do not have details regulations and limits about mycotoxin safe level especially fumonisins. This study later can provide information on setting the new limit for fumonisins safe level thus controls the exposure towards human and animal. To detect accurately the fumonisins production in *F. proliferatum* and *F. verticillioides*, diagnostic method based on polymerase chain reaction (PCR) is the most suitable and rapid detection to detect specific *FUM1* gene pairs as it detects the genes of fumonisins. Since FB_1 contamination in food and feed can lead to health issues in human and animal, the effect is important to be evaluated and assessed to determine the effect of the toxin that can cause health hazard. Therefore, zebrafish is used as a model organism to observe the effect of FB_1 of which it is an ideal choice as it has 70% gene similarity with human genome (Howe et al., 2013). Since currently there are no reports on the exposure of FB_1 on zebrafish, from this study it can provide more information on the effect of FB_1 towards zebrafish which can relate to human health. Comprehensive studies were conducted on FB_1 production by *Fusarium* species, one of the main groups of pathogenic and toxigenic fungi that are frequently found and widely spread throughout the world, including Malaysia.

The objectives of this study were:

i. to identify isolates of *F proliferatum* and *F. verticillioides* based on species-specific PCR assays and evaluate the genetic diversity based on microsatellite markers,
ii. to detect *FUM1* gene and to quantify FB_1 produced by the isolates using UFLC analysis,
iii. to examine the embryotoxicity effect of FB_1 on the larvae of zebrafish (*Danio rerio*).

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REFERENCES

Abbas, H. K., Gelderblom, W. C. A., Cawood, M. E. & Shier, W. T. (1993a). Biological activities of fumonisins, mycotoxins from *Fusarium moniliforme* in jimsonweed (*Datura stramonium* L.) and mammalian cell cultures. *Toxicon, 31,* 345-353.

Abbas, H. K., Duke, S. O. & Tanaka, T. (1993b). Phytotoxicity of fumonisins and related compounds. *Journal of Toxicology and Toxin Review, 12*, 225-251.

Abd Murad, N. B., Mohamed Nor, N. M. I., Shohaimi, S. & Mohd Zainudin, N. A. I. (2017). Genetic diversity and pathogenicity of *Fusarium* species associated with fruit rot disease in banana across Peninsular Malaysia. *Journal of Applied Microbiology. 123*, 1533-1546.

Abdalla, M. Y., Al-Rokibah, A., Moretti, A. & Mule, G. (2000). Pathogenicity of toxigenic *Fusarium proliferatum* from date palm in Saudi Arabia. *Plant Disease*, *84*, 321-324.

Abd-Elsalam, K. A., Moslem, M. & Bakhali, H. A. (2010). Development of di- and tetranucelotide repeat primer for discrimination of *Fusarium* species. *Journal of Food Safety*, *31*, 35-40.

Abu Bakar, A. I., Nur Ain Izzati, M. Z. & Umi Kalsom, Y. (2013). Diversity of *Fusarium* species associated with post-harvest fruit rot disease of tomato. *Sains Malaysiana, 42*, 911-920.

Afsah-Hejri, L., Jinap, S., Hajeb, P., Radu, S. & Shakibazadeh, S. (2013). A review on mycotoxins in food and feed: Malaysia case study. *Comprehensive Reviews in Food Science & Food Safety, 12*, 629-651.

Ahmad, F., Noldus, L. P. J. J., Tegelenbosch, R. A. J. & Richardson, M. K. (2012). Zebrafish embryos and larvae in behavioural assays. *Behaviour, 149,* 1241-1281.

Alessandra, L., Luca, P. & Adriano, M. (2010). Differential gene expression in kernels and silks of maize lines with contrasting levels of ear rot resistance after *Fusarium verticillioides* infection. *Journal of Plant Physiology*, *167*, 1398-1406.

Anthony, S., Abeywickrama, K., Dayananda, R., Wijeratnam, S. W. & Arambewela, L. (2004). Fungal pathogens associated with banana fruit in Sri Lanka and their treatment with essential oils. *Mycopathologia*, *157*, 91-97.

ApSimon, J. W. (2001). Structure, synthesis and biosynthesis of Fumonisin B_1 and related compounds. *Environmental Health Perspectives, 109*, 245-249.

© COPYRIGHT UPM

Asma, A., Shohaimi, S., Noor Baity, S. & Nur Ain Izzati, M. Z. (2018). Characterization and pathogenicity of *Fusarium* species associated with wilt disease of cucumber*. EC Microbiology, 12*, 60-68.

Asran-Amal, A. (2012). Molecular identification of *Macrophomina phaseolina* by microsatellite-based fingerprint. *Plant Pathology & Quarantine, 2*, 143-151.

Bacon, C. W., Porter, J. K., Norred, W. P. & Leslie, J. F. (1996). Production of fusaric acid by *Fusarium* species. *Applied Environmental Microbiology*, *62*, 4039-4043.

Bacon, C. W. & Yates, I. E. (2006). Endophytic root colonization by *Fusarium* species: histology, plant interactions and toxicity. *Microbial Root Endophytes*, *9*, 133-152.

Bahkali, A. H., Abd-Elsalam, K. A., Guo, J. R., Khiyami, M. A. & Verreet, J. A. (2012). Characterization of novel di-, tri-, and tetranucleotide microsatellite primers suitable for genotyping various plant pathogenic fungi with special emphasis on Fusaria and *Mycospherella graminicola. International Journal of Molecular Science, 13*, 2951-2964.

Baird, R., Abbas, H. K., Windham, G., Williams, P., Baird, S., Ma, P., … & Scruggs, M. (2008). Identification of select fumonisin forming *Fusarium* species using PCR applications of the polyketide synthase gene and its relationship to fumonisin production *in vitro*. *International Journal of Molecular Sciences, 9,* 554-570.

Bakos, K., Kovacs, R., Staszny, A., Sipos, D. K., Urbanyi, B., Muller, F., Csenki, Z. & Kovacs, B. (2013). Developmental toxicity and estrogenic potency of zearalenone in zebrafish *(Danio rerio). Aquatic Toxicology, 136-137*, 13-21.

Bane, D. P., Neumann, E. J., Hall, W. F., Harlin K. S. & Slife, R. L. (1992). Relationship between fumonisins contamination of feed and mystery swine disease. A case-control study. *Mycopathologia*, *117,* 121-124.

Barve, M. P., Haware, M. P., Sainani, M. N., Ranjekar, P. K. & Gupta, V. S. (2001). Potential of microsatellites to distinguish four races of *Fusarium oxysporum* f. sp. *ciceri* prevalent in India. *Theoretical and Applied Genetics, 102*, 138-147.

Ben-Ari, G. & Uri, L. (2012). Marker-assisted selection in plant breeding. *Plant Biotechnology and Agriculture, 11,* 163-184.

Bhat, R., Rai, R. V. & Karim, A. (2010). Mycotoxins in food and feed: present status and future concerns. *Comprehensive Reviews in Food Science and Food Safety*, *9*, 57-81.

© COPYRIGHT UPM

Binder, E. M., Tan, L. M., Chin, L. J., Handl, J. & Richard, J. (2007). Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. *Animal Feed and Science Technology, 137*, 265-282.

Bolger, M., Coker, R. D., DiNovi, M., Gaylor, D., Gelderblom, W. & Olsen, M. (2001). The fifty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) Editor. *Safety evaluation of certain mycotoxins in food*. 103-280.

Brown, R., Cleveland, T., Woloshuk, C., Payne, G. A. & Bhatnagar, D. (2001). Growth inhibition of a *Fusarium verticillioides* GUS strain in corn kernels of aflatoxin-resistant genotypes. *Applied Biotechnology and Microbiology*, *57*, 708-711.

Brustein, E., Saint-Amant, L., Buss, R. R., Chong, M., McDearmid, J. R. & Drapeau, P. (2003). Steps during the development of the zebrafish locomotor network. *Journal of Physiology-Paris, 97*, 77-86.

Chakraborty, A., Uechi, T., Higa, S., Torihara, H. & Kenmochi, N. (2009). Loss of ribosomal protein L11 affects zebrafish embryonic development through a p53-dependent apoptotic response. *PLoS One*, e4152.

Chang, K. F., Hwang, S. F., Conner, R. L., Ahmed, H. U., Zhou, Q., Turnbull, G. D., Strelkov, S. E., McLaren, D. L. & Gossen, B. D. (2015). First report of *Fusarium proliferatum* causing root rot in soybean (*Glycine max* L.) in Canada. *Crop Protection. 67*, 52-58.

Chehri, K., Jahromi, S. T., Reddy, K. R. N., Abassi, S. & Baharuddin, S. (2010a). Occurrence of *Fusarium* spp. and fumonisins in stored wheat grains marketed in Iran. *Toxins*, *2*, 2816-2823.

Chehri, K., Abassi, S., Reddy, K. R. N. & Baharuddin, S. (2010b). Occurrence and pathogenicity of various pathogenic fungi on cucurbits from Kermanshah province, Iran. *African Journal of Microbiological Research*, *4*, 1215-1222.

Chehri, K., Salleh, B., Yli-Matilla, T., Reddy, K. R. N. & Abbasi, S. (2011). Molecular characterization of pathogenic *Fusarium* species in cucurbit plants from Kermanshah province, Iran. *Saudi Journal of Biological Sciences, 18*, 341-351,

Cheng, F., Wang, M., Zheng, Y., Luo, J. M., Yang, X. R. & Wang, X. L. (2010). Quantitative changes of plant defense enzymes and phytohormone in biocontrol of cucumber *Fusarium* wilt by *Bacillus subtilis* B579. *World Journal Microbiology*, *26*, 675-684.

Choi, J. H., Lee, S., Nah, J. Y., Kim, H. K., Paek, J. S., Lee, S., … & Lee, T. (2018). Species composition of and fumonisin production by the *Fusarium fujikuroi* species complex isolated from Korean cereals. *International Journal of Food Microbiology. 267*, 62-69.

© COPYRIGHT UPM

Clements, M. J., Maragos, C. M., Pataky, J. K. & White, D. G. (2004). Source of resistance to fumonisin accumulation in grain and fusarium ear and kernel rot of corn. *Phytopathology, 94*, 251-260.

Corley-Smith, G. E., Brandhorst, B. P., Walker, C. & Postlethwait, J. H. (1999). Production of haploid and diploid androgenetic zebrafish (including methodology for delayed in vitro fertilization). *Methods in Cell Biology, 59*, 45-60.

Cummings, M. P. (2004). PAUP* Phylogenetic Analysis Using Parsimony and other methods. Dictionary of Bioinformatics and Computational Biology.

Darnetty, D. M. M. N., Nur Ain Izzati, M. Z., Nor Azliza, I., Nik Mohd Izham, M. N. & Baharuddin, S. (2008). Diversity of *Fusarium* species associated with ear rot of corn in Indonesia and Malaysia. *Proceeding of 6th Regional IMT-GT UNINET Conference* (pp. 422-425).

De Souza, J. & Formento, N. (2004). Studies of anthesis in wheat and its relationship with scab (*Fusarium graminearum* and *Fusarium* spp.). *VI National Congress of Wheat. Fourth National Symposium on Crop Planting Autumn-Winter* (pp. 211-212).

Desjardins, A. E., Plattner, R. D. & Gordon, T. R. (2000). *Gibberella fujikuroi* mating population A and *Fusarium subglutinans* from teosinate species and maize from Mexico and Central America. *Mycology Research*, *104*, 865-872.

Dieringer, D. & Schlotterer, C. (2003). Two distinct modes of microsatellite mutation processes: evidence from the complete genomic sequences of nine species. *Genome Research, 13*, 2242-2251.

Dissanayake, M. L. M. C., Tanaka, S. & Ito, S. (2009). Fumonisin B_1 production by *Fusarium proliferatum* strains isolated from *Allium fistulosum* plants and seeds in Japan. *Letters in Applied Microbiology*, *48*, 598-604.

Doehlert, D. C., Knutson, C. A., & Vesonder, R. E. (1994). Phytotoxic effects of fumonisin B_1 on maize seedling growth. *Mycopathologia, 127*, 117-121.

Dohnal, V., Jezkova, A., Polisenska, I. & Kuca, K. (2010). Determination of fumonisins in milled corn grains using HPLC-MS. *Journal of Chromatographic Science, 48*, 680-684.

Doohan, F. M., Brennan, J. & Cooke, B. M. (2003). Influence of climatic factors on *Fusarium* species pathogenic to cereals. *European Journal of Plant Pathology*, *109*, 755-768.

Duan, C., Qin, Z., Yang, Z., Li, W., Sun, S., Zhu, Z. & Wang, X. (2016). Identification of pathogenic *Fusarium* spp. causing maize ear rot and potential mycotoxin production in China. *Toxins, 8*, 186.

© COPYRIGHT UPM

Egel, D. S. & Martyn, R. D. (2007). Fusarium wilt of watermelon and other cucurbits. *The Plant Health Instructor*, *10*, 1094.

Ellegren, H. (2000). Microsatellite mutations in the germline: implications for evolutionary inference. *Trends Genetics*, *16*, 551-558.

Fandohan, P., Hell, K., Marasas, W. F. O. & Wingfield, M. J. (2003). Infection of maize by *Fusarium* species and contamination with fumonisins in Africa. *African Journal of Biotechnology*, *2*, 570-579.

Faria, C. B., Abe, C. A. L., da Silva, C. N., Tessmann, D. J. & Barbosa-Tessmann, I. P. (2012). New PCR assays for the identification of *Fusarium verticillioides, Fusarium subglutinans* and other species of the *Gibberella fujikuroi* complex. *International Journal of Molecular Science, 13,* 115-132.

Fishman, M. C., Stainier, D. Y., Breitbart, R. E. & Westerfield, M. (1997). Genetic and embryological methods in a transparent vertebrate embryo. *Methods in Cell Biology, 52,* 67-82.

Galvez, L., Urbaniak, M., Waskiewicz, A., Stepien, L. & Palmero, D. (2017). *Fusarium proliferatum*- causal agent of garlic bulb rot in Spain: genetic variability and mycotoxin production. *Food Microbiology, 67,* 41-48.

Garcia, D., Barros, G., Chulze, S., Ramos, A. J., Sanchis, V. & Marin, S. (2012). Impact of cycling temperatures on *Fusarium verticillioides* and *Fusarium graminearum* growth on mycotoxins production in soybean. *Journal of the Science of Food and Agriculture, 92*, 2952-2959.

Garcia, G. R., Noyes, P. D. & Tanguay, R. L. (2016). Advancements in zebrafish applications for 21st century toxicology. *Pharmacology & Therapeutics, 161*, 11-21.

Geiser, D. M., Jimenez-Gasco, M. M., Kang, S., Makalowska, I., Veeraraghavanan, N., Ward, T. J., … & O'Donnell, K. (2004). FUSARIUM-ID v. 1.0: a DNA sequence database for identifying *Fusarium. European Journal of Plant Pathology, 110*, 473-479.

Ghiasian, S. A., Rezayat, S. M., Kord-Bacheh, P., Maghsood, A. H., Shephard, G. S., Westhuizen, L., … & Marasas, W. F. O. (2005). Fumonisins production by *Fusarium* species isolated from freshly harvested corn in Iran. *Mycopathologia*, *159*, 31-40.

Glenn, A. E., Zitomer, N. C., Zimeri, A, M., Williams, L. D., Riley, R. T. & Proctor, R. H. (2008). Transformation-mediated complementation of a *FUM* gene cluster deletion in *Fusarium verticillioides* restores both fumonisin production and pathogenicity on maize seedlings. *Molecular Plant Microbe Interactions, 21*, 87-97.

© COPYRIGHT UPM

Go, K., Horikawa, Y., Garcia, R. & Villarreal, F. J. (2008). Fluorescent method for detection of cleaved collagens using O-phthaldialdehyde (OPA). *Journal of Biochemical and Biophysical Methods, 70*, 878-882.

Gonzalez-Jaen, M. T., Mirete, S., Patino, B., Lopez-Errasquin, E. & Vazquez, C. (2004). Genetic markers for the analysis of variability and for production of specific diagnostic sequences in fumonisins-producing strains of *Fusarium verticillioides. European Journal of Plant Pathology*, *110*, 525-532.

Hanke, N., Staggs, L., Schroder, P., Littarel, J., Heig, S., Kaufeld, J., Pauli, C., Haller, H. & Schiffer, M. (2013). Zebrafishing for novel genes relevant to the glomerular filtration barrier. *BioMed Research International*, 1-12.

Haq, M., Gonzalez, N., Mintz, K., Jaja-Chimedza, A., De Jesus, C. L., Lydon, C., Welch, A. Z. & Berry, P. (2016). Teratogenicity of ochratoxin A and the degradation product, ochratoxin α, in the zebrafish (*Danio rerio*) embryo model of vertebrate development. *Toxins, 8*, 40.

Heng, M. H., Baharuddin, S. & Latiffah, Z. (2011). Molecular identification of *Fusarium* species in *Gibberella fujikuroi* species complex from rice, sugarcane and maize from Peninsular Malaysia. *International Journal of Molecular Sciences, 12*, 6722-6732.

Herbrecht, R., Kessler, R., Kravanja, C. Meyer, M. H., Waller, J. & Lechster-Bru, V. (2004). Successful treatment of *Fusarium proliferatum* pneumonia with prosaconazole in a lung transplant recipient. *Journal of Heart Lung Transplant*, *23*, 1451-1455.

Hill, A. J., Bello, S. M., Prasch, A. L., Peterson, R. E. & Heideman, W. (2004). Water permeability and TCDD-induced edema in zebrafish early-life stages. *Toxicological Science, 78*, 78-87.

Hill, A. J., Teraoka, H., Heideman, W. & Peterson, R. E. (2005). Zebrafish as a model vertebrate for investigating chemical toxicity. *Toxicological Sciences, 86*, 6-19.

Hill, A. J., Heiden, T. C., Heideman, W. & Peterson, R. E. (2009). Potential roles of arnt2 in zebrafish larval development. *Zebrafish, 6*, 79-91.

Hirata, T., Kimishima, E., Aoki, T., Nirenberg, H. I. & O'Donnell, K. (2001). Morphological and molecular characterization of *Fusarium verticillioides* from rotten banana imported into Japan. *Mycoscience, 42*, 155-166.

Hirata, H., Saint-Amant, L., Downes, G. B., Cui, W. W., Zhou, W., Granato, M. & Kuwada, J. Y. (2005). Zebrafish bandoneon mutants display behavioral defects due to a mutation in the glycine receptor β-subunit. *PNAS, 102*, 8345-8350.

© COPYRIGHT UPM

Hopmans, E. C. & Murphy, P. A. (1995). Fumonisins: Mycotoxins produced by *Fusarium species*. Lancaster, Basel. Technocomic Publication & Incorporation. pp. 61–78.

Hosseinzadeh-Colagar, A., Haghighatnia, M. J., Amiri, Z., Mohadjerani, M. & Tafrihi, M. (2016). Microsatellite (SSR) amplification by PCR usually led to polymorphic bands: evidence which shows replication slippage occurs in extend or nascent DNA strands. *Molecular Biology Research Communication, 5*, 167-174.

Howard, P. C., Eppley, R. M., Stack, M. E., Warbritton, A., Voss, K. A., Lorentzen, R. J., … & Bucci, T. J. (2001). Fumonisins B_1 carcinogenicity in two-year feeding study using F344 rats and B6C3F1 mice. *Environmental Health Perspective*, *109*, 277-282.

Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., … & Yong, G. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature, 496,* 498-503.

Huang, T. S., Lee, C. C., Chang, A. C., Lin, S., Chao, C. C., Jou, Y. S., … & Whang-Pheng, J. (2003). Shortening of microsatellite deoxy (CA) repeats involved in GL331 induced down-regulation of matrix metalloproteinase-9 gene expression. *Biochemical and Biophysical Research Communications*, *30*, 901-907.

IARC (2012). Aflatoxins. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 100,* 225-248.

IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY:IBM Corp

Isagi, Y., Kanazashi, T., Suzuki, W., Tanaha, H. & Abe, T. (2000). Microsatellite analysis of the regeneration process of *Magnolia obovate. Heredity*, *84*, 143-161.

Ismaiel, A. A. & Papenbrock, J. (2015). Mycotoxins: producing fungi and mechanisms of phytotoxicity. *Agriculture, 5*, 492-537.

Jackowiak, H., Packa, D., Wiwart, M., Perkowski, J., Busko, M. & Borusiewicz, A., (2002). Scanning electron microscopy of mature wheat kernels infected with *Fusarium culmorum. Journal of Applied Genetics, 43,* 167-176.

Jagadeeswaran, P., Gregory, M., Johnson, S. & Thankavel, B. (2000a). Haemostatic screening and identification of zebrafish mutants with coagulation pathway defects: an approach to identifying novel haemostatic genes in man. *British Journal of Haemotology, 110*, 946-956.

© COPYRIGHT UPM

Jagadeeswaran, P., Gregory, M., Zhou, Y., Zon, L., Padmanabhan, K. & Hanumanthaiah, R. (2000b). Characterization of zebrafish full-length prothrombin cDNA and linkage group mapping. *Blood Cells, Molecules & Diseases, 26*, 479-489.

Jahan-Quazi, S. A., Sariah, M., Hawa, J. & Zainal Abidin, B. M. A. (2013). Characterization of *Fusarium proliferatum* through species specific primers and its virulence on rice seeds. *International Journal of Agriculture and Biology*, *15*, 649-656.

Jany, J. L., Bosquet, J., Gagne, A. & Khasa, D. P. (2006). Simple sequence repeat (SSR) markers in the ectomycorrhizal fungus *Laccaria bicolor* for environmental monitoring of introduced strains and molecular ecology application. *Mycological Research, 110*, 51-59.

Jimenez, M., Mateo, J. J., Hinojo, M. J. & Mateo, R. (2003). Sugars and amino acids as factors affecting the synthesis of fumonisins in liquid cultures by isolates of the *Gibberella fujikuroi* complex. *International Journal of Food Microbiology, 89*, 185-193.

Jurado, M., Marin, P., Magan, N. & Gonzalez-Jaen, M. T. (2008). Relationship between solute, matric potential stress, temperature, growth and *FUM1* gene expression in two *Fusarium verticillioides* strains from Spain. *Applied and Environmental Microbiology, 74*, 2032-2036.

Kalueff, A. V., Gebhardt, M., Stewart, A. M., Cachat, J. M., Brimmer, M., Chawla, J. S., ... & Schneider, H. (2013). Towards a comprehensive catalog of zebrafish behaviour 1.0 and beyond. *Zebrafish, 10*, 70-86.

Karaoglu, H., Man, Y. L. & Meyer, W. (2004). Survey of simple sequence repeats in completed fungal genomes. *Society for Molecular Biology and Evoution*, *22*, 639-649.

Kellerman, T. S., Marasas, W. F. O., Thiel, P. G., Gelderblom, W. C. A., Cawood, M. & Coetzer, J. A. W. (1990). Leukoencephalomalacia in two horses induced by oral dosing of fumonisins B_1. *The Onderstepoort Journal of Veterinary Research, 57*, 269-275.

Kemp, C. D. & Conte, J. V. (2012). The pathophysiology of heart failure. Cardiovascular *Pathology, 21*, 365-371.

Khanzada, K. A., Rajput, M. A., Shah, G. S., Lodhi, A. M. & Mehboob, F. (2002). Effect of seed dressing fungicides for the control of seed borne mycoflora of wheat. *Asian Journal of Plant Science*, *1*, 441-444.

Kleczewski, N. M. & Egel, D. S. (2011). A diagnostic guide for Fusarium wilt of watermelon. *Plant Health Program*. pp. 532-538.

Kohut, G., Adam, A. L., Fazekas, B. & Hornok, L. (2009). N-starvation stress induced *FUM* gene expression and fumonisin production is mediated

© COPYRIGHT UPM

via the HOG-type MAPK pathway in *Fusarium proliferatum. International Journal of Food Microbiology, 130*, 65-69.

Kopf, P. G. & Walker, M. K. (2009). Overview of developmental heart defects by dioxins, PCBs and pesticides. *Journal of Environmental Science and Health. Part C, Environmental Carcinogenesis & Ecotoxicology Reviews, 27*, 276-285.

Kritzinger, Q., Aveling, T. A. S. & van der Merwe, C. F. (2006). Phytotoxic effects of fumonisin B_1 on cowpea seed. *Phytoparasitica, 34,* 178-186.

Kushiro, M., Zheng, Y., Sagaou, Y., Tanaka, K. & Nagata, T. (2007). Liquid chromatographic determination of fumonisins B_1, B_2 and B_3 in rice. *Mycotoxins*, *57*, 95-104.

Kushiro, M., Nagata, R., Nakagawa, H. & Nagashima, H. (2008). Liquid chromatographic detection of fumonisins in rice seed. *National Food Research, 72*, 37-44.

Kwasna, H., Chelkowski, J. & Zajkowski, P. (1991). Flora Polska Mycota. Ploska Akademia Nauk, Institute Botany: Warsaw Poland.

Lamprecht, S. C., Marasas, W. F. O., Alberts, J. F., Cawood, M. E., Gelderblom, W. C. A. & Shephard, G. S. (1994). Phytotoxicity of fumonisins and TA-toxin to corn and tomato. *Phytopathology, 84*, 383-391

Lanubile, A., Pasini, L. & Marocco, A. (2010). Differential gene expression in kernels and silks of maize line with contrasting levels of ear rot resistance after *Fusarium verticillioides* infection. *Journal of Plant Physiology, 167,* 1398-1404.

Lee, S. H., Kim, J. H., Son, S. W., Lee, T. & Yun, S. H. (2012). Fumonisin production by field isolates of the *Gibberella fujikuroi* species complex and *Fusarium commune* obtained from rice and corn in Korea. *Research in Plant Disease, 18*, 310-316.

Leslie, J. F. & Summerell, B. A. (2006). The Fusarium laboratory manual. APS Press.

Leyva-Madrigal, K. Y., Larralde-Corona, C.P., Calderon-Vazquez, C. L. & Maldonado-Mendoza, I. E. (2014). Genome distribution and validation of novel microsatellite markers of *Fusarium verticillioides* and their transferability to other *Fusarium* species. *Journal of Microbiological Methods*, *101*, 18-23.

Lim, S. (1964). Suspected groundnut poisoning in pigs. *Journal of Malaya Medical Society*, *3*, 51.

Lin, Y. H., Chang, J. Y., Liu, E. T., Chao, C. P., Huang, J. W. & Chang, P. F. L. (2009). Development of a molecular marker for specific detection of

© COPYRIGHT UPM

Fusarium oxysporum f. sp. *cubense* race 4. *European Journal of Plant Pathology*, *123*, 353-365.

Lo Magro, S., Campaniello, M., Nardiello, D. & Muscarella, M. (2011). Assessment of fumonisins B_1 and B_2 levels in commercial maize-based food products by liquid chromatography with fluorimetric detection and postcolumn chemical derivatization. *Journal of Food Science, 76,* 1-4.

Logrieco, A., Mule, G., Moretti, A. & Bottalico, A. (2002). Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe. *European Journal of Plant Pathology*, *108*, 597-609.

Lu, G., Cannon, P. F., Reid, A. & Simmons, C. M. (2004). Diversity and molecular relationships of endophytic *Colletotrichum* isolates from the Iwokrama Forest Reserve, Guyana. *Mycological Research, 108*, 53-63.

Madania, A, Altawil, M., Naffaa, W., Holker, P. H. & Hawat, M. (2013). Morphological and molecular characterization of *Fusarium* isolated from maize in Syria. *Journal of Phytopathology, 161*, 452-458.

Magnoli, C. E., Saenz, M. A., Chiacchiera, S. M. & Dalcero, A. M. (1999). Natural occurrence of *Fusarium* species and fumonisin-production by toxigenic strains from poultry feeds in Argentina. *Mycopathologia, 145*, 35-41.

Maharajan, K., Muthulakshmi, S., Nataraj, B., Ramesh, M. & Kadirvelu, K. (2018). Toxicity assessment of pyriproxyfen in vertebrate model zebrafish embryos (*Danio rerio*): a multi biomarker study. *Aquatic Toxicology, 196*, 132-145.

Maheshwar, P. K. & Janardhana, G. R. (2010). Natural occurrence of toxigenic *Fusarium proliferatum* on paddy (*Oryza sativa* L.) in Karnataka, India. *Tropical Life Sciences Research*, *21*, 1-10.

Mahfooz, S., Maurya, D. K., Srivasava, A. K., Kumar, S. & Arora, D. K. (2011). A comparative in-silico analysis on frequency and distribution of microsatellites in coding regions of three formae speciales of *Fusarium oxysporum* and development of EST-SSR markers for polymorphism studies. *FEMS Microbiology Letters, 328*, 54-60.

Marasas, W. F. O. (2001). Discovery and occurrence of the fumonisins: a historical perspective. *Environmental Health Perspective*, *109*, 239-243.

Marasas, W. F. O., Riley, R. T., Hendricks, K. A., Stevens, V. L., Sadler, T. W., Waes, J. … & Merrill, A. H. (2004). Fumonisins disrupt sphingolipid metabolism, folate transport and neural tube development in embryo culture and in vivo: a potential risk factor for human neural tube defects among population consuming fumonisins-contaminated maize. *The Journal of Nutrition*, *134*, 711-716.

© COPYRIGHT IPM

Maschietto, V., Colombi, C., Pirona, R., Pea, G., Strozzi, F., Marocco, A., … & Lanubile, A. (2017). QTL mapping and candidate genes for resistance to Fusarium ear rot and fumonisin contamination maize. *Plant Biology, 17,* 20.

Matthews, M., Trevarrow, B. & Matthews, J. (2002). A virtual tour of the guide for zebrafish users. *Lab Animal, 31*, 34-40.

Medina, A., Schimdt-Heydt, M., Cardenas-Chavez, D. L., Parra, R., Geisen, R. & Magan, N. (2013). Integrating toxin gene expression, growth and fumonisin B_1 and B_2 production by a strain of *Fusarium verticillioides* under different environmental factors. *Journal of Royal Society Interface, 10,* 20130320.

Merrill Jr., A. H., Sullards, M. C., Wang, E., Voss, K. A. & Riley, R. T. (2001). Sphingolipid metabolism: roles in signal transduction and disruption by fumonisins. *Environmental Health Perspectives*, *109*, 283-289.

Miller, J. D. (2001). Factors that affect the occurrence of fumonisins. *Environmental Health Perspective*, *109*, 321-324.

Moges, A. D., Admassu, B., Belew, D., Yesuf, M., Njuguna, J., Kyalo, M. & Ghimire, S. R. (2016). Development of microsatellite markers and analysis of genetic diversity and population structure of *Colletotrichum gloeosporioides* from Ethiopia. *PLOS One, 11*, e0151257.

Moller, E. M., Chelkowski, J. & Geiger, H. H. (1999). Species-specific PCR assays for the fungal pathogens *Fusarium moniliforme* and *Fusarium subglutinans* and their application to diagnose maize ear rot disease. *Journal of Phytopathology*, *147*, 497-508.

Molnar, O., Bartok, T. & Szecsi, A. (2015). Occurrence of *Fusarium verticillioides* and *Fusarium musae* on banana fruits marketed in Hungary. *Acta Microbiologica et Immunologica Hungarica*, *62*, 109-119.

Momol, T., Ji, P., Pernezny, K., McGovern, R. & Olson, S. (2008). Three soil borne tomato diseases caused by *Ralstonia* and *Fusarium* species and their field diagnostics. USA: p. 205. Plant Pathology Department, Florida Coorperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Moncrief, I., Garzon, C., Marek, S., Stack, J., Gamliel, A., Garrido, P., … & Fletcher, J. (2016). Development of simple sequence repeat (SSR) markers for discrimination among isolates of *Fusarium proliferatum*. *Journal of Microbiological Methods*, *126*, 12-17.

Morgante, M., Hanafey, M. & Powell, W. (2002). Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. *Nature Genetics*, *30*, 194-200.

© COPYRIGHT UPM

Moss, M. O. (2012). Mycotoxin review *Aspergillus* and *Penicillium. Mycologist*, *16*, 116-119.

Mukanga, M., Derera, J., Tongoona, P. & Laing, M. D. (2010). A survey of pre-harvest ear rot diseases of maize and associated mycotoxins in south and central Zambia. *International Journal of Food Microbiology*, *141*, 213-221.

Mule, G., Susca, A., Stea, G. & Moretti, A. (2004). A species-specific PCR assay based on the calmodulin partial gene for identification of *Fusarium verticillioides, F. proliferatum* and *F. subglutinans. European Journal of Plant Pathology*, *110*, 495-502.

Munkvold, G. P. & Carlton, W. M. (1997). Influence of inoculation method on systemic *Fusarium moniliforme* infection of maize plants grown from infected seeds. *Plant Diseases, 81*, 211-216.

Munkvold, G. P. (2003). Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. *European Journal of Plant Pathology*, *109*, 705-713.

Murillo-Williams, A. & Munkvold, G. P. (2008). Systemic infection by *Fusarium verticillioides* in maize plants grown under three temperature regimes. *Plant Disease, 92,* 1695-1700.

Nelson, S. (2008). Postharvest rots of banana. Plant Disease. University of Hawaii.

Nor Azizah, K. (2012). *Fusarium* sp. associated with diseased ginger plants in Penang Island. Degree Thesis. Universiti Sains Malaysia.

Nor Azliza, I., Masratul Hawa, M., Nik Mohd Izham, M. N. & Latiffah, Z. (2017). Fumonisin B_1-producing *Fusarium* species from agricultural crops in Malaysia. *Crop Protection, 98*, 70-75.

Norred, W. P., Bacon, C. W., Riley, R. T., Voss, K. A. & Meredith F. I. (1999). Screening of fungal species for fumonisins production and fumonisin-like disruption of sphingolipid biosynthesis. *Mycopathologia*, *146*, 91-98.

Nur Adni, M. (2014). Molecular characterization of endophytic fungi isolated from *Eurycoma longifolia* jack and Malay traditional vegetables. Degree Thesis. Universiti Putra Malaysia.

Nur Ain Izzati, M. Z., Azmi, A. R. & Baharuddin, S. (2008). Secondary metabolite profiles and mating populations of *Fusarium* species in section *Liseola* associated with bakanae disease of rice. *Malaysian Journal of Microbiology, 4*, 6-13.

© COPYRIGHT UPM

Nur Ain Izzati, M. Z., Siti Nordahliawate, M. S., Nor Azliza, I. and Baharuddin, S. (2009). Distribution and diversity of *Fusarium s*pecies associated with grasses in Peninsular Malaysia. *Biotropika*, *16*, 55-64.

Nur Ain Izzati, M. Z. & Baharuddin, S. (2010). Variability of *Fusarium* species associated with Bakanae disease of rice based on their virulence, vegetative and biological compatibilities. *Sydowia*, *62*, 89-104.

Nur Ain Izzati, M. Z., Siti Nordahliawate, M. S., Nor Azlina, J., Azmi, A. R. & Baharuddin, S. (2011a). Isolation and identification of *Fusarium* species associated with Fusarium ear rot disease of corn. *Pertanika Journal of Tropical Agricultural Science*, *34*, 325-330.

Nur Ain Izzati M. Z., Azmi A. R., Atta M. A. A. & Norazlina J. (2011b). Contribution to the knowledge of diversity of *Fusarium* associated with maize in Malaysia. *Plant Protection Science*, *47*, 20.

Nur Ain Izzati, M. Z. & Nithiyaa, P. (2015). Mycotoxins production by *Fusarium* and *Aspergillus* species isolated from cornmeal. *International Journal of Microbiology of Agriculture & Biology, 17*, 440-448.

Nur Baiti, A. M., Nor Azizah, K. & Nur Ain Izzati, M. Z. (2016). Identification and diversity of *Fusarium* species isolated from tomato fruits. *Journal of Plant Protection Research, 56*, 231-236.

Nurul Farizah, A. (2014). Morphological identification and molecular characterization of *Fusarium proliferatum* (Matsushima, Nirenberg) in Malaysia. Master Science Thesis. Universiti Sains Malaysia.

Nur Hazrati, M., Hafizi, R., Nor Azliza, I, Baharuddin, S. & Latiffah, Z. (2012). Diveristy of *Fusarium* species from highland areas in Malaysia. *Tropical Life Sciences Research, 23*, 1-15.

Nurul Huda, M. S. & Latiffah, Z. (2014). Occurrence of *Fusarium* spp. on vegetable crops and assessment of their pathogenicity. *Tropical Agricultural Science. 37*, 445-455.

Nurul Husna, O., Masratulhawa, M., Nik Mohd Izham, M. N. & Latiffah, Z. (2018). Characterization and pathogenicity of *Fusarium* species associated with leaf spot of mango (*Mangifera indica* L.). *Microbial Pathogenesis, 114*, 362-368.

Oladiran, A. O. & Iwu, L. N. (1992). Changes in ascorbic acid and carbohydrate contents in tomato fruits infected with pathogens. *Plant Foods for Human Nutrition, 42,* 373-382.

Paggi, G. M., Palma-Silva, C., Bered, F., Cidade, F. W., Sousa, A. C. B., Souza, A. P., … & Lexer, C. (2008). Isolation and characterization of microsatellite loci in *Pitcairnia albiflos* (Bromeliaceae), an endemic

© COPYRIGHT UPM

bromeliad from the Atlantic Rainforest, and cross-amplification in other species. *Molecular Ecology Resources*, *8*, 980-982.

Palmero, D., De Cara, M., Nosir, W., Galvez, L., Cruz, A., Woodward, S., … & Tello J. C. (2012). *Fusarium proliferatum* isolated from garlic in Spain: identification, toxigenic potential and pathogenicity on related *Allium* species. *Phytopathologia Mediterranea*, *51*, 207-218.

Patino, B., Mirete, S., Gonzalez-Jaen, T., Mule, G., Rodriguez, M. T. & Vazquez, C. (2004). PCR detection assays of fumonisin-producing *Fusarium verticillioides* strains. *Journal of Food Protection*, *67*, 1278-1283.

Pavlovic, S., Ristic, D., Vucurovic, I., Stevanovic, M., Stojanovic, S., Kuzmanovic, S. & Starovic, M. (2016). Morphology, pathogenicity and molecular identification of *Fusarium* spp. associated with anise seeds in Serbia. *Not Bot Horti Agrobo, 44*, 411-417.

Peakall, R. & Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population and research-an update. *Bioinformatics*, *28,* 2537-2539.

Picot, A., Barreau, C., Pinson-Gadais, L., Caron, D., Lannou, C. & Richard-Forget, F. (2010). Factors of the *Fusarium verticillioides*-maize environment modulation fumonisin production. *Critical Reviews in Microbiology, 36*, 221-231.

Pivonia, S., Cohen, R., Kafkafi, U., Ben, Z. I. S. & Katan, J. (1997). Sudden wilt of melons in southern Israel: fungal agents and relationship with plant development. *Plant Diseases, 81,* 1264-1268.

Presello, D. A., Iglesias, J., Botta, G. & Eyherabide, G. H. (2007). Severity of Fusarium ear rot and concentration of fumonisin in grain of Argentinian maize hybrids. *Crop Protection*, *26*, 852-855.

Proctor, R. H., Desjardins, A. E., Plattner, R. D. & Hohn, T. M. (1999). A polyketide synthase gene required for biosynthesis of fumonisin mycotoxins in *Gibberella fujikuroi* mating population A. *Fungal Genetics and Biology, 27*, 100-112.

Proctor, R. H., Brown, D. W., Plattner, R. D. & Desjardins, A. E. (2003). Co-expression of 15 contiguous genes delineates a fumonisin biosynthetic gene cluster in *Gibberella moniliformis. Fungal Genetics and Biology, 38,* 237-249.

Proctor, R. H., Plattner, R. D., Brown, D. W., Seo, J. A. & Lee, Y. W. (2004). Discontinuous distribution of fumonisin biosynthetic genes in the *Gibberella fujikuroi* species complex. *Mycological Research, 108*, 815-822.

© COPYRIGHT UPM

Qiu, J., Xu, J., Dong, F., Yin, X. & Shi, J. (2015). Isolatation and characterization of *Fusarium verticillioides* from maize in eastern China. *European Journal of Plant Pathology, 142,* 791-800.

Rahjoo, V., Zad, J., Javan-Nikkah, M., Mizardi Gohari, A., Okhovvat, S. M., Bihamta, M. R., Razzaghian, J. & Klemsdal, S. S. (2008). Morphological and molecular identification of *Fusarium* isolated from maize ears in Iran. *Journal of Plant Pathology, 90*, 463-468.

Rampersad, S. N., Perez-Brito, D., Torres-Calzada, C., Tapia-Tussell, R. & Carrington, C. V. F. (2013). Genetic structure of *Colletotrichum gloeosporioides* sensu lato isolates infecting papaya inferred by multilocus ISSR markers. *Phytopathology, 103,* 182-189.

Reddy, K. R. N. & Salleh, B. (2011). Co-occurrence of moulds and mycotoxins in corn grains used for animal feeds in Malaysia. *Journal of Animal and Veterinary Advances*, *10*, 668-673.

Reid, L. M., Zhu, X. & Ma, B. L. (2001). Crop rotation and nitrogen effects on maize susceptibility to *Gibberella* (*Fusarium graminearum*) ear rot. *Plant and Soil*, *237*, 1-14.

Ren, Y., Zhao, H., Kou, Q., Jiang, J., Guo, S., Zhang, H., … & Xu, Y. (2012). A high resolution genetic map anchoring scaffolds of the sequenced watermelon genome. PLoS ONE, *7*, e29453.

Rheeder, J. P., Marasas, W. F. O. & Vismer, H. F. (2002). Production of fumonisins analogs by *Fusarium species. Applied and Environmental Biology, 68*, 2101-2105.

Richard, E., Heutte, N., Sage, L., Pottier, D., Bouchart, V., Lebailly, P. & Garon, D. (2007). Toxigenic fungi and mycotoxins in mature corn silage. *Food and Chemical Toxicology*, *45*, 2420-2425.

Rockman, M. V. & Wray, G. A. (2002). Abundant raw material for cis-regulatory evolution in humans. *Molecular Biology and Evolution*, *19*, 1991-2004.

Russell, L., Cox, D. F., Larsen, G., Bodwell, K. & Nelson C. E. (1991). Incidence of moulds and mycotoxins in commercial animal feed mills in seven Midwestern states 1998-1989. *Journal of Animal Science*, *69*, 5-12.

Saharan, M. S. & Naef, A. (2008). Detection of genetic variation among Indian wheat head scab pathogens (*Fusarium* spp./isolates) with microsatellite markers. *Crop Protection*, *27*, 1148-1154.

Samapundo, S., Devliehgere, F., De Meulenaer, B. & Debevere, J. (2005). Effect of water activity and temperature on growth and the relationship between fumonisin production and the radial growth of *Fusarium*

© COPYRIGHT UPM

verticillioides and *Fusarium proliferatum* on corn. *Journal of Food Protection, 68*, 1054-1059.

Saraswathy, N. & Ramalingam, P. (2011). Genome mapping. *Concepts and Techniques in Genomics and Proteomics*, pp. 77-93.

Scauflaire, J., Mahieu, O., Louvieaux, J., Foucart, G., Renard, F. & Munaut, F. (2011). Biodiversity of *Fusarium* species in ears and stalks of maize plants in Belgium. *European Journal of Plant Pathology, 131*, 59.

Scherz, P. J., Huisken, J., Sahai-Hernandez, P. & Stainier, D. Y. (2008). High-speed imaging of developing deart valves reveals interplay of morphogenesis and function. *Development, 135*, 1179-1187.

Schmidt-Heydt, M., Magan, N. & Geisen, R. (2008). Stress induction of mycotoxin biosynthesis genes by abiotic factors. *FEMS Microbiology Letters, 284*, 142-149.

Seo, J. A., Kim, J. C. & Lee, Y. W. (1999). N-acetyl derivatives of type C fumonisins produced by *Fusarium oxysporum. Journal of National Product*, *62*, 355-357.

Seo, Y. & Kim, Y. H. (2017). Potential reasons for prevalence of Fusarium wilt in oriental melon in Korea. *Plant Pathology Journal, 33*, 249-263.

Sharma, K. D., Winter, P., Kahl, G. & Muehlbauer, F. J. (2004). Molecular mapping of *Fusarium oxysporum* f. sp. *ciceris* race 3 resistance gene in chickpea. *Theory of Applied Genetics*, *108*, 1243-1248.

Shephard, G. S. (1998). Chromatographic determination of the fumonisins mycotoxins. *Journal of Chromatography A, 815*, 31-39.

Singh, R., Sheoran, S., Sharma, P. & Chatrath, R. (2011). Analysis of simple sequence repeats (SSRs) dynamics in fungus *Fusarium graminearum. Bioinformation, 5,* 402-404.

Siti Nordahliawate, M. S. (2007). Pathogenicity and aethiology of *Fusarium* species associated with Pokkah Boeng disease on sugarcane. Master Science Thesis. Universiti Sains Malaysia.

Slatkin, M. (1985). Gene flow in natural populations. *Annual Review in Ecological System, 16*, 393-430.

Smith, L. E., Stoltzfus, R. J. & Prendergast, A. (2012). Food chain mycotoxin exposure, gut health and impaired growth: a conceptual framework. *Advances in Nutrition, 3*, 526-531.

Soleimany, F., Jinap, S., Rahmani, A. & Khatib, A. (2011). Simultaneous detection of 12 mycotoxins in cereals using RP-HPLC-PDA-FLD with

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PHRED and a post-column derivatization system. *Food Additives and Contaminants*, *28*, 494-501.

Soleimany, F., Jinap, S. & Abas, F. (2012a). Determination of mycotoxins in cereals by liquid chromatography tandem mass spectrometry. *Food Chemistry, 130*, 1055-1060.

Soleimany, F., Jinap, S., Faridah, A. & Khatib, A. (2012b). A UPLC-MS/MS for simultaneous determination of aflatoxins, ochratoxin A, zearalenone, DON, fumonisins, T-2 toxin and HT- toxin in cereals. *Food Control*, *25*, 647-653.

Soriano, J. M., Gonzalez, L. & Catala, A. I., (2005). Mechanism of action of sphingolipids and their metabolites in the toxicity of fumonisin B_1. *Progress in Lipid Research, 44*, 345-356.

Spitsbergen, J. M. & Kent, M. L. (2003). The state of the art of the zebrafish model for toxicology and toxicologic pathology research-advantages and current limitations. *Toxicologic Pathology, 31*, 62-87.

Sreenivasa, M. Y., Dass, R. S., Raj, A. P. C. & Janardhana, G. R. (2006). Molecular detection of fumonisin producing *Fusarium* species of freshly harvested maize kernels using Polymerase Chain Reaction (PCR). *Taiwania, 51*, 251-257.

Srobarova, A., Moretti, A., Ferracane, R., Ritieni, A. & Logrieco, A. (2002). Toxigenic *Fusarium species* of *Liseola* section in preharvest maize ear rot and associated mycotoxins in Slovakia. *European Journal of Plant Pathology*, *108*, 299-306.

Summerell, B. A., Baharuddin, S. & Leslie, J. F. (2003). A utilitarian approach to *Fusarium* identification. *Plant Disease*, *87*, 117-128.

Toth, G., Gaspari, Z. & Jurka, J. (2000). Microsatellites in different eukaryotic genome: survey and analysis. *Genome Research*, *10*, 1967-1981.

Tournas, V. H. & Katsoudas, E. (2005). Mould and yeast flora in fresh berries, grapes and citrus fruits. *International Journal of Food Microbiology, 105*, 11-17.

Triest, D., Pierard, D., De Cremer, K. & Hendrickx, M. (2016). *Fusarium musae* infected banana fruits as potential source of human fusariosis: may occur more frequently than we might think and hypotheses about infection. *Communicative & Integrative Biology, 9*, e1162934.

Tsai, Y. J., Pan, H., Hung, C. M., Hou, P. T., Li, Y. C., Lee, Y. J., … & Li, C. (2011). The predominant protein arginine methyltransferase PRMT1 is critical for zebrafish convergence and extension during gastrulation. *The FEBS Journal, 278*, 905-917.

© COPYRIGHT UPM

Uddin, M. S. & Cheng, Q. (2015). Recent application of biotechniques for the improvement of mango research. *Applied Plant Genomics and Biotechnology, 12,* 195-212.

Van Hove, F., Waalwijk, C., Logrieco, A., Munaut, F. & Moretti, A. (2011). *Gibberella musae* (*Fusarium musae*) sp. nov., a revently discovered species from banana is sister to *F. verticillioides*. *Mycologia, 103*, 570-585.

Vogelsang, S., Widmer, F., Jenny, E. & Enkerli, J. (2009). Characterization of novel *Fusarium graminearum* microsatellite markers in different *Fusarium species* from various countries. *European Journal of Plant Pathology*, *123*, 477-482.

Vogelsang, S., Enkerli, J., Jenny, E., Roffler, S. & Widmer, F. (2011). Characterization of *Fusarium poae* microsatellite markers on strains from Switzerland and other countries. *Journal of Phytopathology*, *159*, 197-200.

Voss, K. A., Riley, R. T., Norred, W. P., Bacon, C. W., Meredith, F. I., Howard, P. C., … & Porter, J. K. (2001). An overview of rodent toxicities: liver and kidney effects of fumonisins and *Fusarium moniliforme. Envormental Health Perspectives, 109*, 259-266.

Walsh, P. S., Fildes, N. J. & Reynolds, R. (1996). Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus. *Nucleic Acid Research, 24*, 2807-2812.

Wang, J., Wang, X., Zhou, Y., Du., L. & Wang, Q. (2010). Fumonisin detection and analysis of potential fumonisin-producing *Fusarium* spp. in asparagus (*Asparagus officinalis* L.) in Zhejiang Province of China. *Journal of the Science of Food and Agriculture, 90*, 836-842.

Weidenborner, M. (2001). Foods and fumonisins. *European Food Research and Technology*, *212*, 262-273.

WHO/FAO (2001). Safety evaluation of certain mycotoxins in food. WHO Food Addictive Series 47/FAO Food and Nutrition Paper. Geneva; WHO.

Wu, T. S., Yang, J. J., Yu, F. Y. & Liu, B. H. (2013). Cardiotoxicity of mycotoxin citrinin and involvement of microRNA-138 in zebrafish embryos. *Toxicological Sceinces, 136*, 402-412.

Wu, T. S., Yang, J. J., Wang, Y. W., Yu, F. Y. & Liu, B. H. (2016). Mycotoxin ochratoxin A disrupts renal development via miR-731/prolactin receptor axis in zebrafish. *Toxicology Research, 5*, 519-529.

Wu, T. S., Lin, Y. T., Huang, Y. T., Cheng, Y. C., Yu, F. Y. & Liu, B. H. (2018). Disruption of liver development and coagulation pathway by ochratoxin A in embryonic zebrafish. *Toxicology and Applied Pharmacology. 340*, 1-8.

© COPYRIGHT UPM

Yang, Y., Qi, S., Wang, D., Wang, K., Zhu, L., Chai, T. & Wang C. (2016). Toxic effects of thifluzamide on zebrafish (*Danio rerio*). *Journal of Hazardous Materials, 307*, 127-136.

Yin, E. S., Rakhmankulova, M., Kucera, K., de Sena Filho, J. G., Portero, C. E., Narvaes-Trujillo, A., … & Strobel, S. A. (2015). Fusaric acid induces a notochord malformation in zebrafish via copper chelation. *Biometals, 28*, 783-789.

Yogeshwar, D. K., Noelle, S., Hile, S. E., Chiaromonte, F., Eckert, K. A. & Makova, K. D. (2010). What is a microsatellite: a computational and experimental definition based upon repeat mutation behaviour at A/T and GT/AC repeats. *Genome Biology and Evolution*, *2*, 620-635.

Yuan, G., Wang, Y., Yuan, X., Zhang, T., Zhao, J., Huang, L. & Peng, S. (2014). T-2 toxin induces developmental toxicity and apoptosis in zebrafish embryos. *Journal of Environmental Sciences, 26,* 917-925.

Zain, M. E. (2011). Impacts of mycotoxins on humans and animals. *Journal of Saudi Chemical Society*, *15*, 129-144.

Zhang, F., Wan, X. Q. & Tan G. T. (2006). QTL mapping of *Fusarium moniliforme* ear rot resistance in maize, 1. Map construction with microsatellite and AFLP markers. *Journal of Applied Genetics*, *47*, 9-15.

Zhang, S., Raza, W., Yang, X., Hu, J., Huang, Q. & Liu, X. (2008). Control of *Fusarium* wilt disease of cucumber plants with the application of a bioorganic fertilizer. *Biology and Fertilizer of Soil, 44*, 1073-1080.

Zheng, Q. & Ploetz, R. (2002). Genetic diversity in the mango malformation pathogen and development of a PCR assay. *Plant Pathology*, *51*, 208-216.

Zhou, H., George, S., Li, C, Gurusamy, S., Sun, X., Gong, Z. & Qian, H. (2017). Combined toxicity of prevalent mycotoxins studied in fish cell line and zebrafish larvae revealed that type of interaction is dose-dependent. *Aquatic Toxicology. 193*, 60-71.

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